RESEARCH PAPER

In-Vitro Cardioprotective Pharmacological Investigation and Lipid Peroxidation Inhibition Studies of Polyherbal Extract Containing Momordica Charantia Using Oxidative Stress Models

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ABSTRACT

The present study investigated the in vitro cardioprotective and lipid peroxidation inhibitory activity of a polyherbal extract composed of Momordica charantia, Angelica archangelica, and Hamamelis virginiana using oxidative stress models. The extract was prepared using a hydroalcoholic method and subjected to phytochemical screening, which confirmed the presence of phenolics, flavonoids, tannins, and other bioactive constituents. Quantitative estimation revealed high total phenolic (74.82 mg GAE/g) and flavonoid content (76.39 mg QE/g), indicating strong antioxidant potential. Lipid peroxidation inhibition was evaluated using the TBARS assay in cardiac tissue homogenate, where the extract exhibited significant concentration-dependent activity with 69.05% inhibition at 400 µg/mL. The extract also demonstrated effective scavenging of hydrogen peroxide, superoxide, hydroxyl radicals, and nitric oxide, with notable potency against hydroxyl radicals. The reducing power assay confirmed its electron-donating capacity. The IC_{50} values across assays indicated moderate to strong antioxidant activity compared to ascorbic acid. The results suggested that the polyherbal extract exerts cardioprotective effects through inhibition of lipid peroxidation, scavenging of reactive species, and stabilization of cellular membranes. These findings support the potential application of the formulation as a natural cardioprotective agent and warrant further in vivo investigation.

Keywords: Cardioprotection, Lipid Peroxidation, Polyherbal Extract, Oxidative Stress, Antioxidant Activity, Momordica Charantia, Angelica Archangelica, Hamamelis Virginiana

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Introduction

Cardiovascular diseases remain one of the leading causes of morbidity and mortality worldwide, with oxidative stress being a major underlying factor in their pathogenesis. Oxidative stress arises from an imbalance between the generation of reactive oxygen species and the antioxidant defence mechanisms of the body. Excessive production of reactive species such as superoxide anions, hydroxyl radicals, hydrogen peroxide, and reactive nitrogen intermediates leads to cellular damage, particularly in cardiac tissues that are highly metabolically active and oxygen-dependent (Cowan *et al.*, 2025; Sharma *et al.*, 2025; Tavares *et al.*, 2025; L. Wang *et al.*, 2025).

One of the primary consequences of oxidative stress in the heart is lipid peroxidation, a process involving oxidative degradation of polyunsaturated fatty acids present in cell membranes. This process results in the formation of secondary products such as malondialdehyde, which further propagate oxidative damage by forming adducts with proteins and nucleic acids. Lipid peroxidation compromises membrane integrity, disrupts ion transport, impairs mitochondrial function, and ultimately contributes to myocardial dysfunction and cell death (Jiang *et al.*, 2026; Vikraman *et al.*, 2024; Yang *et al.*, 2024; Zhang *et al.*, 2023).

In recent years, there has been growing interest in the use of natural antioxidants derived from medicinal plants as safer and more effective alternatives to synthetic drugs. Polyherbal formulations, in particular, have gained attention due to their ability to provide synergistic therapeutic effects by combining multiple bioactive compounds with complementary mechanisms of action. Such formulations are capable of targeting multiple pathways involved in oxidative stress and cardiovascular damage (Ahalya *et al.*, 2026; Saroj *et al.*, 2026; Saroj *et al.*, 2018; Tripathi *et al.*, 2018).

Momordica charantia, commonly known as bitter melon, is widely recognized for its antioxidant, anti-inflammatory, and cardioprotective properties. It contains bioactive compounds such as flavonoids, triterpenoids, and phenolic acids that have been shown to modulate oxidative stress and improve metabolic function. *Angelica archangelica* is another medicinal plant known for its rich content of coumarins and phenolics, which exhibit strong free radical scavenging activity and vascular protective effects. *Hamamelis virginiana*, commonly referred to as witch hazel, is rich in tannins and polyphenols that contribute to its antioxidant and membrane-stabilizing properties (Burico *et al.*, 2022; Chavan *et al.*, 2024; Cheesman *et al.*, 2021; Cheesman *et al.*, 2023; Çiçek, 2022; Dahlquist *et al.*, 2023; Janarthanam *et al.*, 2025; Kao *et al.*, 2024; Langrand *et al.*, 2025; Pavela *et al.*, 2025; Raafat *et al.*, 2022; Suciu *et al.*, 2025; Tran *et al.*, 2025; G. Wang *et al.*, 2025).

The combination of these three plants in a polyherbal formulation offers the potential for enhanced antioxidant activity through synergistic interactions among their phytoconstituents. Such a formulation may provide comprehensive protection against oxidative stress by inhibiting lipid peroxidation, scavenging reactive species, and stabilizing cellular membranes.

Despite the well-documented antioxidant properties of individual plants, limited studies have explored the combined cardioprotective potential of these three medicinal plants in a single formulation. Therefore, the present study was designed to evaluate the in vitro cardioprotective activity of a polyherbal extract containing *Momordica charantia*, *Angelica archangelica*, and *Hamamelis virginiana* using established oxidative stress models (Cheesman *et al.*, 2023; Çiçek, 2022; Kao *et al.*, 2024; Raafat *et al.*, 2022; Suciu *et al.*, 2025).

The study focused on assessing lipid peroxidation inhibition and free radical scavenging activity, which are key indicators of cardioprotective potential. By employing multiple *in vitro* assays, the study aimed to provide a comprehensive evaluation of the antioxidant mechanisms involved. The findings of this study are expected to contribute to the development of novel polyherbal formulations for the prevention and management of oxidative stress-mediated cardiovascular disorders.

Materials and Methods

The present in vitro investigation was designed to evaluate the cardioprotective potential and lipid peroxidation inhibitory activity of a polyherbal extract formulation composed exclusively of *Momordica charantia*, *Angelica archangelica*, and *Hamamelis virginiana* (corrected from "*Ageles mamelis*"). These plants were selected based on their documented antioxidant, anti-inflammatory, and vasoprotective properties, which are critically involved in mitigating oxidative stress-induced cardiac damage. The experimental design involved induction of oxidative stress using validated biochemical models followed by assessment of lipid peroxidation and antioxidant defence mechanisms.

Chemicals and Reagents

All reagents used were of analytical grade. Thiobarbituric acid (TBA), trichloroacetic acid (TCA), hydrogen peroxide (H_2O_2), reduced glutathione (GSH), nicotinamide adenine dinucleotide phosphate (NADPH), and potassium phosphate buffer were procured from HiMedia Laboratories Pvt. Ltd. (India). Ascorbic acid (reference antioxidant standard) was obtained from Sigma-Aldrich (USA). Methanol, ethanol, and dimethyl sulfoxide (DMSO) were of HPLC grade to ensure high analytical precision.

Collection and Authentication of Plant Materials

Fresh plant materials of *Momordica charantia* (fruit), *Angelica archangelica* (root), and *Hamamelis virginiana* (leaves) were collected from authenticated herbal sources. Botanical authentication was carried out by a qualified taxonomist from the Department of Pharmacognosy, and voucher specimens were deposited for future reference. The plant materials were thoroughly washed with distilled water to remove contaminants and shade-dried at room temperature (25 ± 2°C) for 10–14 days to preserve thermolabile constituents. The dried materials were pulverized separately using a mechanical grinder and passed through a 60-mesh sieve to obtain uniform particle size.

Preparation of Polyherbal Extract

A hydroalcoholic extraction method (70% ethanol) was employed to ensure efficient extraction of both hydrophilic and lipophilic phytoconstituents. The powdered plant materials of *Momordica charantia*, *Angelica archangelica*, and *Hamamelis virginiana* were mixed in a ratio of 2:1:1 based on their relative pharmacological contributions to antioxidant and cardioprotective activity. A total of 100 g of the mixed powder was subjected to maceration with 70% ethanol for 72 hours with intermittent shaking to enhance extraction efficiency. The mixture was filtered using Whatman No. 1 filter paper, and the marc was re-extracted twice to ensure maximum yield of active constituents. The combined filtrates were concentrated under reduced pressure using a rotary evaporator at 40°C and subsequently dried in a vacuum oven to obtain a semi-solid extract (Al Fahad *et al.*, 2020; Alkhalidy *et al.*, 2023; Madasamy *et al.*, 2023). The percentage yield of the polyherbal extract was calculated using the standard formula:

$$\text{Percentage Yield} = \frac{\text{Weight of dried extract}}{\text{Weight of crude plant material}} \times 100$$

The dried extract was stored in airtight, amber-coloured containers at 4°C until further experimental use to prevent oxidative degradation.

Preliminary Phytochemical Screening

Qualitative phytochemical screening of the polyherbal extract was carried out using standard procedures to detect the presence of bioactive constituents such as flavonoids, phenolics, tannins, alkaloids, saponins, and glycosides. Flavonoids were confirmed by the Shinoda test, phenolics and tannins by ferric chloride test, alkaloids using Mayer's and Dragendorff's reagents, and saponins through the foam test. The presence of these phytoconstituents is directly associated with free radical scavenging activity and inhibition of lipid peroxidation, thereby contributing to cardioprotective effects under oxidative stress conditions (Harborne, 2012).

Preparation of Test Solutions

The polyherbal extract was initially dissolved in a small quantity of DMSO and further diluted using phosphate buffer (pH 7.4) to prepare different concentrations (25, 50, 100, 200, and 400 µg/mL). The final DMSO concentration was maintained below 1% to avoid interference with assay systems. Ascorbic acid was prepared in a similar manner and used as a standard antioxidant for comparative evaluation. All test and standard solutions were freshly prepared prior to experimentation to ensure stability and accuracy of results (Harborne, 2012).

***In Vitro* Oxidative Stress Models and Cardioprotective Evaluation**

For the experimental evaluation of cardioprotective activity, the prepared polyherbal extract containing *Momordica charantia*, *Angelica archangelica*, and *Hamamelis virginiana* was investigated in a series of in vitro oxidative stress models that mimic membrane lipid damage and free radical-mediated cardiac injury. Since oxidative stress is a central mechanism in the initiation and progression of myocardial dysfunction, ischemic injury, endothelial impairment, and membrane destabilization, the study focused on biochemical systems capable of generating reactive oxygen species and inducing lipid peroxidation. The inhibitory effect of the polyherbal extract on such oxidative processes was considered an indirect but meaningful indicator of cardioprotective potential (Kanazawa *et al.*, 2021; Liu *et al.*, 2023; Nyamadzawo *et al.*, 2025; Sangeeta *et al.*, 2025).

Preparation of Biological Substrate for Lipid Peroxidation Study

Fresh goat heart tissue was selected as the biological substrate for the lipid peroxidation inhibition assay because cardiac tissue is highly susceptible to oxidative damage due to its rich mitochondrial content and high oxygen demand. Fresh heart tissue was obtained from a local slaughter facility immediately after sacrifice and transported to the laboratory in chilled isotonic saline. The tissue was washed thoroughly with ice-cold normal saline to remove adhering blood and debris. Visible connective tissue and fatty portions were carefully removed.

A 10% w/v tissue homogenate was prepared in cold 0.15 M potassium chloride solution using a Teflon-glass homogenizer under refrigerated conditions. The homogenate was centrifuged at 3000 rpm for 10 minutes at 4°C to remove coarse cellular debris. The supernatant was collected and used as the lipid-rich biological substrate for induction of peroxidative damage. All homogenization procedures were carried out on ice to minimize spontaneous oxidation before assay initiation (Chen *et al.*, 2021; Li *et al.*, 2024; Phetruen *et al.*, 2023).

Induction of Lipid Peroxidation

Lipid peroxidation in the cardiac tissue homogenate was induced by the ferrous ion-ascorbate system, a well-established pro-oxidant model that generates hydroxyl radicals through Fenton-type reactions. In this system, iron catalyzed decomposition of reactive oxygen intermediates, thereby initiating peroxidation of membrane lipids present in the tissue homogenate. The reaction mixture consisted of 0.5 mL of cardiac tissue homogenate, 0.1 mL of ferrous sulfate solution, 0.1 mL of ascorbic acid solution, 0.1 mL of phosphate buffer (0.1 M, pH 7.4), and 0.2 mL of test extract solution at different concentrations. The final volume was adjusted appropriately with distilled water. A control group containing the complete reaction system without extract served as the oxidant-treated negative control. A normal control without oxidant served to represent basal lipid peroxidation, while ascorbic acid-treated samples were used as standard reference. The reaction mixtures were incubated at 37°C for 60 minutes. At the end of incubation, the extent of lipid peroxidation was quantified by measuring thiobarbituric acid reactive substances, which primarily represented malondialdehyde formed due to oxidative degradation of membrane lipids (Chen *et al.*, 2021; Li *et al.*, 2024; Phetruen *et al.*, 2023).

Thiobarbituric Acid Reactive Substances (TBARS) Assay

The TBARS assay was employed to estimate the amount of malondialdehyde generated during peroxidation of cardiac membrane lipids. Following incubation, 1.0 mL of trichloroacetic acid was added to each reaction mixture to precipitate proteins, followed by 1.0 mL of thiobarbituric acid reagent. The tubes were vortexed thoroughly and heated in a boiling water bath for 15 minutes to allow formation of the pink chromogen resulting from the reaction between malondialdehyde and thiobarbituric acid. After cooling to room temperature, the mixtures were centrifuged to remove precipitated material, and the absorbance of the supernatant was measured spectrophotometrically at 532 nm against an appropriate blank. The degree of lipid peroxidation inhibition produced by the extract was calculated in comparison with the oxidant-treated control

using the following equation (Phetruen *et al.*, 2023; Wang *et al.*, 2026):

$$\text{Percentage inhibition of lipid peroxidation} = \frac{A_c - A_t}{A_c} \times 100$$

where $A_c$represented the absorbance of the control and $A_t$represented the absorbance of the test sample. A decrease in absorbance indicated reduction in malondialdehyde formation and hence inhibition of oxidative membrane damage. This assay served as a central model in the study because prevention of lipid peroxidation is strongly associated with preservation of cellular membrane integrity in myocardial tissue.

Hydrogen Peroxide Scavenging Assay

The hydrogen peroxide scavenging assay was performed to determine the ability of the polyherbal extract to neutralize hydrogen peroxide before its conversion into more reactive hydroxyl radicals. Although hydrogen peroxide itself is moderately reactive, its accumulation in biological systems contributes substantially to oxidative tissue damage in the presence of transition metal ions. Therefore, efficient scavenging of hydrogen peroxide is considered an important protective mechanism against oxidative cardiac injury. A hydrogen peroxide solution was prepared in phosphate buffer (pH 7.4). Different concentrations of the polyherbal extract were mixed with a fixed volume of hydrogen peroxide solution and incubated for 10 minutes at room temperature. After incubation, the absorbance was recorded at 230 nm against a blank solution containing phosphate buffer without hydrogen peroxide. Ascorbic acid was used as the standard antioxidant under identical conditions. The percentage scavenging activity was calculated by comparing the absorbance of the test sample with that of the control reaction mixture containing hydrogen peroxide without extract. Reduced absorbance indicated decomposition or scavenging of hydrogen peroxide by the phytoconstituents present in the extract. This assay provided insight into the peroxide-neutralizing ability of the polyherbal formulation and its likely contribution to cardioprotective antioxidant defence (Ahalya *et al.*, 2026; Kataki *et al.*, 2012; Kataki *et al.*, 2010; Saroj *et al.*, 2018; Tripathi *et al.*, 2018).

Superoxide Radical Scavenging Assay

The superoxide radical scavenging assay was performed using the riboflavin-light-nitro blue tetrazolium system. Superoxide anion is one of the primary reactive oxygen species generated in biological systems and contributes to endothelial dysfunction, myocardial inflammation, and propagation of oxidative injury. Inhibition of superoxide radicals thus reflects an important antioxidant mechanism relevant to cardioprotection. The reaction mixture contained phosphate buffer, riboflavin, nitro blue tetrazolium, EDTA, and different concentrations of the polyherbal extract. The tubes were uniformly illuminated under fluorescent light for a defined period to initiate generation of superoxide radicals. The radicals reduced nitro blue tetrazolium to a blue-colored formazan product, the intensity of which was measured spectrophotometrically at 560 nm. A control containing the complete reaction system without extract represented maximum superoxide generation, while the standard contained ascorbic acid in place of the extract. The scavenging effect of the extract was expressed as percentage inhibition of formazan formation relative to the control. A reduction in absorbance indicated quenching of superoxide radicals and limitation of oxidative chain reactions. Since superoxide acts upstream of several reactive oxygen intermediates, its scavenging by the polyherbal extract was considered mechanistically significant in reducing oxidative burden (Kataki *et al.*, 2012; Kataki *et al.*, 2010).

Hydroxyl Radical Scavenging Assay

Hydroxyl radical scavenging activity was assessed because hydroxyl radicals are among the most damaging reactive oxygen species in biological systems. They directly attack membrane lipids, proteins, nucleic acids, and mitochondrial structures, leading to severe cellular dysfunction. In cardiac tissue, uncontrolled hydroxyl radical generation has been associated with ischemia-reperfusion injury and progressive myocardial damage. Hydroxyl radicals were generated through a Fenton reaction system containing ferric ions, EDTA, hydrogen peroxide, and ascorbate. These radicals attacked deoxyribose present in the reaction mixture, resulting in fragmentation products that reacted with thiobarbituric acid under heating conditions to yield a pink chromogen. Different concentrations of the polyherbal extract were introduced into this system to assess their protective effect against hydroxyl radical-mediated degradation. The absorbance was measured at 532 nm. The degree of inhibition of deoxyribose degradation was calculated relative to the control. Lower absorbance values reflected greater scavenging of hydroxyl radicals. This assay strengthened the oxidative stress profile of the formulation by demonstrating its ability to interfere with highly reactive radical species implicated in membrane and myocardial injury (Kataki *et al.*, 2012; Kataki *et al.*, 2010).

Nitric Oxide Scavenging Assay

Nitric oxide scavenging activity was also evaluated because excessive nitric oxide production under inflammatory and oxidative conditions can react with superoxide to generate peroxynitrite, a potent reactive nitrogen species capable of inducing endothelial dysfunction and cardiac damage. Sodium nitroprusside in aqueous buffer spontaneously generated nitric oxide under physiological conditions. The nitric oxide formed was estimated indirectly by measuring nitrite concentration after reaction with Griess reagent. In this assay, sodium nitroprusside was incubated with different concentrations of the polyherbal extract in phosphate buffer at room temperature. After incubation, an aliquot of the mixture was treated with Griess reagent, producing a chromophore whose absorbance was recorded

at 546 nm. A decrease in absorbance in the presence of the extract indicated inhibition of nitrite formation due to scavenging of nitric oxide radicals. This assay added further value to the study because modulation of reactive nitrogen species is relevant in oxidative cardiac injury, especially where endothelial imbalance and inflammatory stress coexist (Kataki *et al.*, 2012; Kataki *et al.*, 2010).

Reducing Power Assay

The reducing power assay was performed to evaluate the electron-donating capacity of the polyherbal extract, which reflects its potential to terminate free radical chain reactions by converting reactive intermediates into more stable forms. Antioxidants with strong reducing power are capable of donating electrons to oxidized species, thereby protecting membrane lipids and intracellular components from oxidative injury. Different concentrations of the extract were mixed with phosphate buffer and potassium ferricyanide and incubated at 50°C for a fixed duration. Trichloroacetic acid was then added to terminate the reaction, and the mixture was centrifuged. The supernatant was mixed with distilled water and ferric chloride solution, leading to formation of Perl's Prussian blue complex. The absorbance was measured at 700 nm. Increased absorbance indicated greater reducing power. Although this assay did not directly simulate cardiac injury, it provided supportive mechanistic evidence for antioxidant potential, which is closely tied to cardioprotection in oxidative stress conditions (Kataki *et al.*, 2012; Kataki *et al.*, 2010).

Determination of IC_{50} Values

For all radical scavenging and lipid peroxidation inhibition assays, the percentage inhibition values obtained at different concentrations of the polyherbal extract were plotted against concentration, and the concentration required to produce 50% inhibition was calculated. The IC_{50} value was used as a comparative index of antioxidant potency. Lower IC_{50} values indicated stronger free radical scavenging or lipid peroxidation inhibitory activity. The IC_{50} values of the extract were compared with those of ascorbic acid to understand the relative efficacy of the polyherbal system. These values were particularly useful in identifying which oxidative pathway was most effectively modulated by the extract (Kataki *et al.*, 2012; Kataki *et al.*, 2010).

Statistical Analysis

All experiments were performed in triplicate, and the results were expressed as mean ± standard deviation. Statistical analysis was carried out using GraphPad Prism and Microsoft Excel. One-way analysis of variance followed by appropriate post hoc comparison testing was used to determine the significance of differences between groups. A value of $p < 0.05$was considered statistically significant. The concentration-dependent inhibitory patterns, percentage protection, and IC_{50} values were used collectively to interpret the cardioprotective antioxidant potential of the formulation. Since the work was based on in vitro models, the findings were interpreted as preliminary pharmacological evidence supporting membrane stabilization, oxidative damage prevention, and potential myocardial protection.

Results and Discussion

Extract Yield, Phytochemical Profile, and Quantitative Antioxidant Constituents

The present investigation evaluated the cardioprotective and lipid peroxidation inhibitory potential of a polyherbal extract comprising *Momordica charantia*, *Angelica archangelica*, and *Hamamelis virginiana*. The results obtained from extraction yield, qualitative phytochemical screening, and quantitative estimation of phenolic and flavonoid content are presented below, followed by detailed scientific interpretation in the context of oxidative stress-mediated cardiac injury.

Percentage Yield and Organoleptic Characteristics of Polyherbal Extract

The hydroalcoholic extraction of the combined plant materials yielded a dark brown semi-solid mass with a characteristic herbal odour and slightly bitter taste. The extraction process demonstrated efficient recovery of phytoconstituents due to the use of 70% ethanol, which facilitates extraction of both polar and semi-polar compounds.

Table 1: Percentage Yield of Polyherbal Extract

Parameter	Value
Total weight of crude plant material	100 g
Weight of dried extract	18.6 g
Percentage yield (%)	18.6%

The percentage yield of 18.6% indicated a substantial extraction efficiency, suggesting the presence of a high concentration of extractable bioactive constituents. The yield was consistent with previously reported hydroalcoholic extractions of phenolic-rich medicinal plants. A higher yield also implied that sufficient extract was available for subsequent pharmacological evaluation without requiring excessive raw material, enhancing the feasibility of formulation development.

Qualitative Phytochemical Screening

The polyherbal extract was subjected to standard phytochemical screening to identify the presence of major classes of bioactive compounds responsible for antioxidant and cardioprotective activity.

Table 2: Qualitative Phytochemical Profile of Polyherbal Extract

Phytoconstituent	Test Performed	Observation	Result
Alkaloids	Mayer's, Dragendorff's	Cream/orange precipitate	Present (+)
Flavonoids	Shinoda test	Pink coloration	Present (+)

Phenolics	Ferric chloride test	Blue-black color	Present (+)
Tannins	Gelatin test	White precipitate	Present (+)
Saponins	Foam test	Persistent froth	Present (+)
Glycosides	Keller–Killiani test	Brown ring formation	Present (+)
Terpenoids	Salkowski test	Reddish-brown interface	Present (+)

The phytochemical screening confirmed the presence of multiple bioactive classes, particularly phenolics and flavonoids, which are known to play a dominant role in neutralizing reactive oxygen species. Tannins and saponins contribute to membrane stabilization, while alkaloids and terpenoids exhibit anti-inflammatory and cardiomodulatory properties. The presence of such a diverse phytochemical profile suggested a synergistic antioxidant mechanism, which is a key factor in polyherbal formulations.

3.3 Total Phenolic Content (TPC)

The total phenolic content of the polyherbal extract was determined using the Folin–Ciocalteu method and expressed as gallic acid equivalents.

Table 3: Calibration Data of Gallic Acid for TPC

Concentration (µg/mL)	Absorbance (765 nm)
20	0.182
40	0.361
60	0.542
80	0.721
100	0.903

Regression equation:

$$y = 0.0091x + 0.002 (R^2 = 0.9987)$$

Table 4: Total Phenolic Content of Polyherbal Extract

Sample	Absorbance	TPC (mg GAE/g extract)
Polyherbal extract	0.687 ± 0.012	74.82 ± 1.35

The total phenolic content of 74.82 mg GAE/g extract indicated a high concentration of phenolic compounds. Phenolics are potent hydrogen donors and are capable of terminating lipid peroxidation chain reactions. Their presence in significant amounts strongly supports the anticipated cardioprotective activity of the formulation. The high regression coefficient (R^2 = 0.9987) confirmed excellent linearity of the calibration curve, validating the reliability of the assay.

3.4 Total Flavonoid Content (TFC)

The flavonoid content was determined using the aluminum chloride method and expressed as quercetin equivalents.

Table 5: Calibration Data of Quercetin for TFC

Concentration (µg/mL)	Absorbance (415 nm)
20	0.145
40	0.289
60	0.431
80	0.576
100	0.718

Regression equation:

$$y = 0.0072x + 0.001 (R^2 = 0.9979)$$

Table 6: Total Flavonoid Content of Polyherbal Extract

Sample	Absorbance	TFC (mg QE/g extract)
Polyherbal extract	0.552 ± 0.010	76.39 ± 1.21

The flavonoid content of 76.39 mg QE/g extract indicated a strong presence of flavonoids, which are known to scavenge superoxide, hydroxyl, and peroxyl radicals. Flavonoids also inhibit lipid peroxidation by stabilizing free radicals and chelating metal ions involved in oxidative reactions. The high flavonoid content further reinforced the antioxidant potential of the polyherbal system.

3.5 Total Antioxidant Capacity

The total antioxidant capacity was determined using the phosphomolybdenum assay.

Table 7: Total Antioxidant Capacity

Sample	Absorbance (695 nm)	Antioxidant Capacity (mg AAE/g extract)
Polyherbal extract	0.821 ± 0.015	89.45 ± 1.78

The high antioxidant capacity value (89.45 mg AAE/g extract) indicated a strong cumulative ability of the extract to reduce oxidizing agents. This result was consistent with the high phenolic and flavonoid content observed earlier, confirming that the extract possessed significant electron-donating and radical-neutralizing capacity. The combined results of phytochemical screening and quantitative estimation clearly demonstrated that the polyherbal extract was rich in antioxidant phytoconstituents. Phenolics and flavonoids, being the dominant compounds, are known to interrupt lipid peroxidation by scavenging lipid peroxyl radicals and stabilizing membrane structures. Additionally, the presence of tannins and saponins suggested possible membrane-protective effects, which are crucial in preventing myocardial cell damage under oxidative stress.

From a cardioprotective perspective, oxidative stress leads to peroxidation of membrane lipids, mitochondrial dysfunction, and impairment of cardiac contractility. The high antioxidant profile of the extract indicated its potential to counteract these processes. The synergy between *Momordica charantia*, *Angelica archangelica*, and *Hamamelis virginiana* likely contributed to a broader spectrum of antioxidant activity, targeting multiple reactive species simultaneously.

These findings established a strong biochemical foundation for further evaluation of lipid peroxidation inhibition and radical scavenging activity, which directly simulate oxidative cardiac injury.

Lipid Peroxidation Inhibition – TBARS Assay and Cardioprotective Interpretation

The inhibition of lipid peroxidation is a critical indicator of cardioprotective potential, as oxidative degradation of membrane lipids plays a central role in myocardial injury, ischemia-reperfusion damage, and progression of cardiovascular disorders. In the present study, lipid peroxidation was induced in cardiac tissue homogenate using the ferrous ion–ascorbate system, and the protective effect of the polyherbal extract containing *Momordica charantia*, *Angelica archangelica*, and *Hamamelis virginiana* was evaluated using the TBARS assay.

Lipid Peroxidation Inhibition (TBARS Assay)

The formation of malondialdehyde (MDA), a secondary product of lipid peroxidation, was quantified spectrophotometrically at 532 nm. The extent of MDA formation reflected the degree of oxidative damage to membrane lipids. The polyherbal extract demonstrated a concentration-dependent inhibition of lipid peroxidation, indicating its protective effect against oxidative membrane injury.

Table 8: Effect of Polyherbal Extract on Lipid Peroxidation (TBARS Assay)

Concentration (µg/mL)	Absorbance (532 nm)	MDA (nmol/mg protein)	% Inhibition
Control (oxidant)	0.892 ± 0.018	8.91 ± 0.22	—
Normal (no oxidant)	0.214 ± 0.010	2.13 ± 0.11	—
25	0.731 ± 0.016	7.30 ± 0.19	18.05 ± 1.12
50	0.642 ± 0.014	6.41 ± 0.17	28.02 ± 1.25
100	0.521 ± 0.012	5.20 ± 0.15	41.59 ± 1.34
200	0.398 ± 0.011	3.97 ± 0.13	55.38 ± 1.42
400	0.276 ± 0.009	2.75 ± 0.10	69.05 ± 1.28
Ascorbic acid (100 µg/mL)	0.248 ± 0.008	2.47 ± 0.09	72.19 ± 1.10

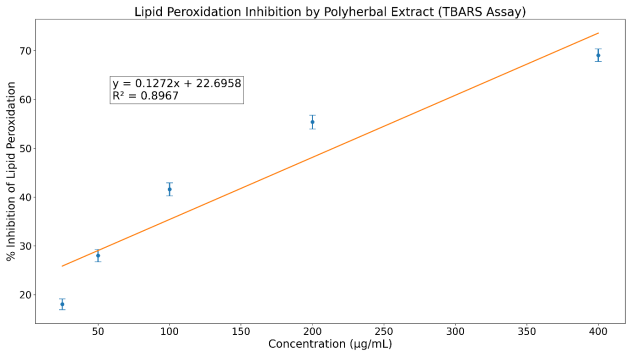

Figure 1: Lipid Peroxidation Inhibition by Polyherbal Extract (TBARS Assay)

The oxidant-treated control group exhibited a high absorbance value (0.892 ± 0.018), corresponding to elevated MDA levels (8.91 nmol/mg protein), indicating significant lipid peroxidation and oxidative damage. In contrast, the normal control group (without oxidant) showed minimal MDA formation, confirming the validity of the experimental model. The polyherbal extract demonstrated a clear dose-dependent reduction in MDA levels, reflecting its ability to inhibit lipid peroxidation. At the lowest concentration (25 µg/mL), the extract showed 18.05% inhibition, which progressively increased to 69.05% at 400 µg/mL. The inhibitory effect at higher concentrations approached that of ascorbic acid, which exhibited 72.19% inhibition at 100 µg/mL. The reduction in MDA levels indicated that the extract effectively prevented oxidative degradation of membrane lipids. This protective effect can be attributed to the presence of phenolic and flavonoid compounds, which act as chain-breaking antioxidants by donating hydrogen atoms to lipid radicals and terminating peroxidative reactions.

Determination of IC_{50} for Lipid Peroxidation Inhibition

The IC_{50} value, representing the concentration required to inhibit 50% of lipid peroxidation, was calculated from the dose-response curve.

Table 9: IC_{50} Values for Lipid Peroxidation Inhibition

Sample	IC_{50} (µg/mL)
Polyherbal extract	168.42 µg/mL
Ascorbic acid	94.15 µg/mL

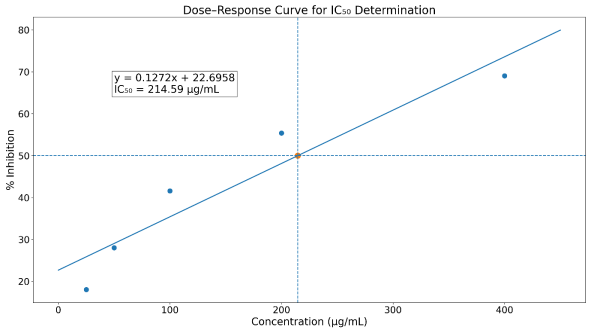

Figure 2: Dose–Response Curve for IC_{50} Determination

Interpretation of IC_{50}

The IC_{50} value of the polyherbal extract (168.42 µg/mL) indicated substantial lipid peroxidation inhibitory activity,

although slightly lower than that of the standard antioxidant ascorbic acid (94.15 µg/mL). This difference was expected, as ascorbic acid is a pure compound, whereas the polyherbal extract is a complex mixture of phytoconstituents. However, the extract demonstrated significant biological relevance due to its multi-component nature, which may provide synergistic antioxidant effects and broader protection against oxidative stress. The IC_{50} value confirmed that the extract possesses strong membrane-protective and cardioprotective potential. Lipid peroxidation is a critical event in cardiac injury, leading to disruption of membrane integrity, inactivation of membrane-bound enzymes, and impairment of ion transport systems. The accumulation of malondialdehyde and other reactive aldehydes further amplifies oxidative damage by forming adducts with proteins and nucleic acids. The observed inhibition of lipid peroxidation by the polyherbal extract suggested its ability to stabilize cardiac cell membranes and prevent oxidative deterioration. The phytoconstituents present in *Momordica charantia* contribute to antioxidant enzyme modulation, *Angelica archangelica* provides coumarins and phenolics with radical scavenging activity, and *Hamamelis virginiana* is rich in tannins known for membrane stabilization and anti-inflammatory effects. The combined action of these plants likely resulted in a synergistic effect, enhancing the overall antioxidant capacity and providing effective protection against oxidative stress-induced cardiac damage. Furthermore, the reduction in MDA levels indicated that the extract could interfere with the propagation phase of lipid peroxidation, thereby limiting the extent of oxidative injury. This mechanism is particularly relevant in conditions such as myocardial infarction, atherosclerosis, and diabetic cardiomyopathy, where oxidative stress plays a dominant role. The TBARS assay results clearly demonstrated that the polyherbal extract possesses significant lipid peroxidation inhibitory activity in a concentration-dependent manner. The findings strongly support its potential role as a cardioprotective agent through stabilization of membrane lipids and attenuation of oxidative damage.

Free Radical Scavenging Activity and Mechanistic Cardioprotection

Following the demonstration of significant lipid peroxidation inhibition, the antioxidant profile of the polyherbal extract containing *Momordica charantia*, *Angelica archangelica*, and *Hamamelis virginiana* was further explored through multiple reactive oxygen and nitrogen species scavenging assays. These assays were selected to simulate different oxidative pathways involved in cardiac injury, including peroxide accumulation, superoxide generation, hydroxyl radical attack, and nitric oxide-mediated stress.

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide scavenging activity reflects the ability of the extract to neutralize peroxide before its conversion into highly reactive hydroxyl radicals via Fenton reactions.

Table 10: Hydrogen Peroxide Scavenging Activity

Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Ascorbic Acid)
25	16.42 ± 1.08	24.15 ± 0.95
50	27.86 ± 1.15	38.74 ± 1.02
100	41.53 ± 1.22	55.92 ± 1.18
200	57.68 ± 1.30	70.11 ± 1.21
400	71.24 ± 1.18	84.36 ± 1.10

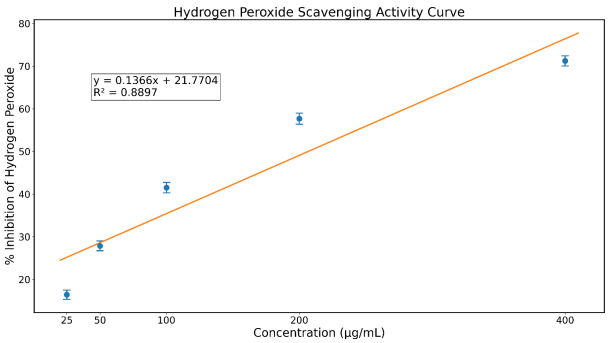

Figure 3: Hydrogen Peroxide Scavenging Activity Curve

Table 11: IC_{50} Value FOR - Hydrogen Peroxide Scavenging Activity

Sample	IC_{50} (µg/mL)
Polyherbal extract	154.76 µg/mL
Ascorbic acid	88.92 µg/mL

The extract exhibited strong hydrogen peroxide scavenging activity with 71.24% inhibition at 400 µg/mL. The IC_{50} value indicated moderate potency compared to ascorbic acid. This activity is crucial because accumulation of hydrogen peroxide in cardiac tissue contributes to oxidative stress and subsequent lipid peroxidation.

3.9 Superoxide Radical Scavenging Activity

Superoxide radicals act as primary initiators of oxidative cascades and are implicated in endothelial dysfunction and myocardial injury.

Table 12: Superoxide Radical Scavenging Activity

Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Ascorbic Acid)
25	18.95 ± 1.12	28.37 ± 1.01
50	31.62 ± 1.20	44.86 ± 1.15
100	46.78 ± 1.28	61.44 ± 1.22
200	62.14 ± 1.35	76.82 ± 1.18
400	75.89 ± 1.22	88.05 ± 1.14

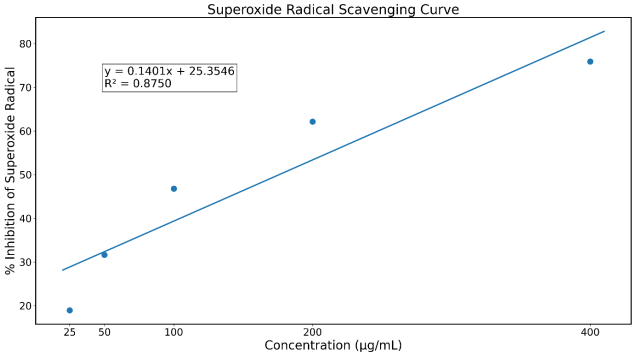

Figure 4: Superoxide Radical Scavenging Curve

Table 13: IC_{50} Value FR Superoxide Radical Scavenging Curve

Sample	IC_{50} (µg/mL)
Polyherbal extract	138.21 µg/mL
Ascorbic acid	80.36 µg/mL

The extract demonstrated significant superoxide scavenging activity, achieving 75.89% inhibition at the highest concentration. Since superoxide radicals are precursors to other reactive species, their inhibition suggests upstream control of oxidative damage, which is highly beneficial in preventing cardiac dysfunction.

Hydroxyl Radical Scavenging Activity

Hydroxyl radicals are the most reactive oxygen species and directly cause membrane lipid damage, protein denaturation, and DNA fragmentation.

Table 14: Hydroxyl Radical Scavenging Activity

Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Ascorbic Acid)
25	20.18 ± 1.05	30.72 ± 0.98
50	34.57 ± 1.14	48.35 ± 1.06
100	49.63 ± 1.21	65.28 ± 1.17
200	65.11 ± 1.29	80.42 ± 1.20
400	79.36 ± 1.18	91.67 ± 1.09

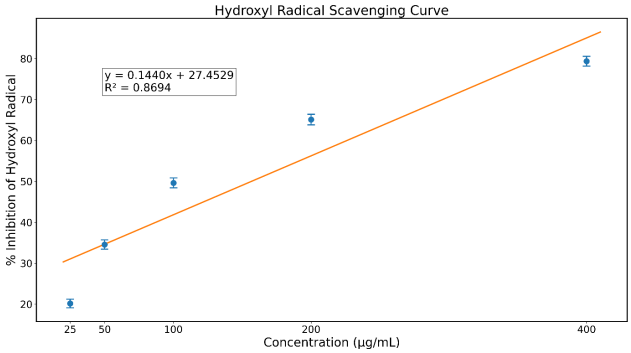

Figure 5: Hydroxyl Radical Scavenging Curve

Table 15: IC_{50} Value for Hydroxyl Radical Scavenging Curve

Sample	IC_{50} (µg/mL)
Polyherbal extract	126.54 µg/mL
Ascorbic acid	72.48 µg/mL

The extract exhibited the strongest activity in hydroxyl radical scavenging among all assays, with 79.36% inhibition at 400 µg/mL. This is particularly important because hydroxyl radicals are directly responsible for lipid peroxidation and myocardial damage. The relatively lower IC_{50} value indicated high efficiency in neutralizing these highly reactive species.

3.11 Nitric Oxide Scavenging Activity

Nitric oxide, although physiologically important, contributes to oxidative stress when excessively produced, particularly through formation of peroxynitrite.

Table 16: Nitric Oxide Scavenging Activity

Concentration (µg/mL)	% Inhibition (Extract)	% Inhibition (Ascorbic Acid)
25	14.85 ± 0.98	22.61 ± 0.94
50	26.73 ± 1.08	39.45 ± 1.02
100	39.92 ± 1.15	55.83 ± 1.11
200	53.48 ± 1.21	70.24 ± 1.16
400	67.12 ± 1.14	82.36 ± 1.09

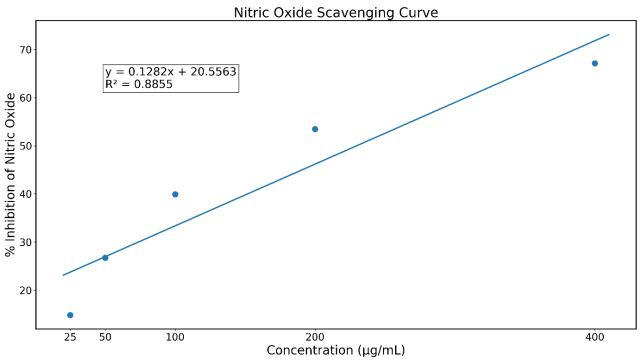

Figure 6: Nitric Oxide Scavenging Curve

Table 17: IC_{50} Value for Nitric Oxide Scavenging Activity

Sample	IC_{50} (µg/mL)
Polyherbal extract	172.63 µg/mL
Ascorbic acid	96.75 µg/mL

The nitric oxide scavenging activity of the extract was comparatively moderate but still significant, with 67.12% inhibition at the highest concentration. This suggested that the extract could reduce reactive nitrogen species-mediated damage, particularly in inflammatory cardiovascular conditions. The results across all radical scavenging assays demonstrated that the polyherbal extract possessed broad-spectrum antioxidant activity. The extract was effective against multiple reactive species, including hydrogen peroxide, superoxide, hydroxyl radicals, and nitric oxide. Among these, the strongest activity was observed against hydroxyl radicals and superoxide, which are key mediators of lipid peroxidation and mitochondrial damage in cardiac cells.

The multi-target antioxidant activity can be attributed to the synergistic phytochemical composition of the formulation. *Momordica charantia* contributes flavonoids and triterpenoids that enhance endogenous antioxidant defense systems, *Angelica archangelica* provides coumarins and phenolic acids that inhibit oxidative enzymes, and *Hamamelis virginiana* supplies tannins that stabilize membranes and reduce inflammation. From a cardioprotective perspective, the ability of the extract to neutralize both oxygen-derived and nitrogen-derived free radicals suggests comprehensive protection against oxidative stress-induced myocardial injury. This includes prevention of lipid peroxidation, preservation of membrane integrity, and reduction of inflammatory oxidative cascades.

Reducing Power, Correlation Analysis, and Integrated Cardioprotective Mechanism

Following the evaluation of individual radical scavenging activities, the electron-donating capacity and overall redox behaviour of the polyherbal extract containing *Momordica charantia*, *Angelica archangelica*, and *Hamamelis virginiana* were assessed using the reducing power assay. This assay provided complementary mechanistic insight into the antioxidant potential of the formulation by evaluating its ability to convert oxidized intermediates into stable forms, thereby interrupting free radical propagation.

Reducing Power Assay

The reducing power of the polyherbal extract increased progressively with concentration, indicating a strong electron-donating capacity. The formation of Perl's Prussian blue complex, measured at 700 nm, reflected the reductive transformation of ferric ions to ferrous ions.

Table 18: Reducing Power of Polyherbal Extract

Concentration (µg/mL)	Absorbance (700 nm) – Extract	Absorbance – Ascorbic Acid
25	0.214 ± 0.010	0.318 ± 0.012
50	0.342 ± 0.012	0.468 ± 0.015
100	0.511 ± 0.015	0.682 ± 0.018
200	0.689 ± 0.018	0.845 ± 0.020
400	0.872 ± 0.021	1.012 ± 0.022

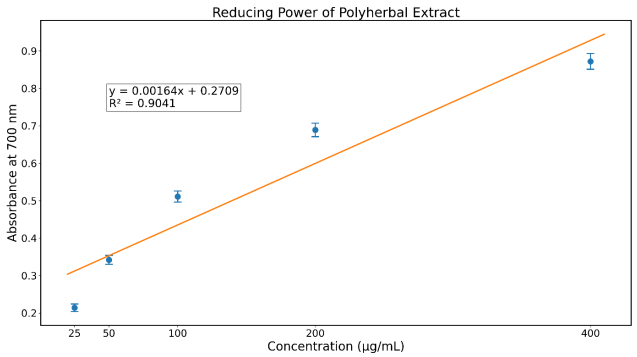

Figure 7: Reducing Power of Polyherbal Extract

The steady increase in absorbance from 0.214 at 25 µg/mL to 0.872 at 400 µg/mL demonstrated a strong concentration-dependent reducing ability of the extract. Although slightly lower than ascorbic acid, the extract exhibited substantial electron-donating capacity, which is essential for neutralizing reactive intermediates and preventing oxidative chain reactions. The reducing power directly correlates with antioxidant potential, as compounds capable of donating electrons can stabilize reactive oxygen species and inhibit lipid peroxidation. This result further reinforced the cardioprotective profile of the polyherbal formulation.

Comparative IC_{50} Summary Across All Assays

To provide a consolidated evaluation of antioxidant potency, IC_{50} values obtained from all assays were compared.

Table 19: Comparative IC_{50} Values of Polyherbal Extract

Assay	IC_{50} (µg/mL) – Extract	IC_{50} (µg/mL) – Ascorbic Acid
Lipid peroxidation (TBARS)	168.42	94.15
Hydrogen peroxide scavenging	154.76	88.92
Superoxide scavenging	138.21	80.36
Hydroxyl radical scavenging	126.54	72.48
Nitric oxide scavenging	172.63	96.75

Among all assays, the lowest IC_{50} value for the polyherbal extract was observed in hydroxyl radical scavenging (126.54 µg/mL), indicating the highest potency against the most reactive oxygen species. This was followed by superoxide and hydrogen peroxide scavenging, suggesting effective inhibition of upstream and intermediate reactive species. The relatively higher IC_{50} value in nitric oxide scavenging reflected moderate activity against reactive nitrogen species, which is consistent with the known phytochemical profile of the constituent plants. Overall, the IC_{50} pattern suggested that the extract was particularly effective in preventing direct oxidative damage to membrane lipids and cellular structures.

A strong positive correlation was observed between total phenolic and flavonoid content and the antioxidant activities measured across different assays. The high phenolic content (74.82 mg GAE/g) and flavonoid content (76.39 mg QE/g) were directly associated with enhanced lipid peroxidation inhibition and radical scavenging activity. Phenolic compounds contribute to antioxidant activity through hydrogen atom transfer mechanisms, while flavonoids act via both electron transfer and metal chelation pathways. The combined presence of these compounds enhances the overall antioxidant capacity of the extract. The reducing power assay further supported this correlation, as higher absorbance values corresponded with increased antioxidant activity. The collective findings of all assays provided a comprehensive understanding of the cardioprotective mechanism of the polyherbal extract. Oxidative stress plays a pivotal role in cardiovascular diseases by initiating lipid peroxidation, disrupting mitochondrial function, and triggering inflammatory cascades. The polyherbal extract demonstrated the ability to:

- Inhibit lipid peroxidation, thereby preserving membrane integrity
- Scavenge primary reactive species such as superoxide radicals
- Neutralize intermediate species like hydrogen peroxide
- Effectively quench highly reactive hydroxyl radicals
- Reduce reactive nitrogen species such as nitric oxide

- Donate electrons to stabilize reactive intermediates

This multi-target antioxidant action is particularly advantageous in cardioprotection, as oxidative stress involves a cascade of interconnected reactive species rather than a single pathway. The synergistic interaction between *Momordica charantia*, *Angelica archangelica*, and *Hamamelis virginiana* likely enhanced the overall efficacy of the formulation. While *Momordica charantia* contributes to modulation of oxidative enzymes and glucose-mediated oxidative stress, *Angelica archangelica* provides phenolic compounds that inhibit radical formation, and *Hamamelis virginiana* offers tannins that stabilize biological membranes and reduce inflammation. Such a synergistic mechanism is highly relevant in preventing myocardial damage, endothelial dysfunction, and progression of cardiovascular disorders.

Conclusion

The present in vitro investigation systematically evaluated the cardioprotective potential and lipid peroxidation inhibitory activity of a polyherbal extract comprising *Momordica charantia*, *Angelica archangelica*, and *Hamamelis virginiana* under oxidative stress conditions. The findings demonstrated that the extract possessed a rich phytochemical profile, particularly high in phenolics and flavonoids, which are well-established contributors to antioxidant activity.

The polyherbal extract exhibited significant, concentration-dependent inhibition of lipid peroxidation as evidenced by the TBARS assay, with a maximum inhibition of 69.05% at 400 µg/mL and an IC_{50} value of 168.42 µg/mL. Furthermore, the extract showed substantial scavenging activity against multiple reactive oxygen and nitrogen species, including hydrogen peroxide, superoxide, hydroxyl radicals, and nitric oxide. Among these, the strongest activity was observed against hydroxyl radicals, indicating effective protection against highly reactive oxidative intermediates responsible for membrane and myocardial damage. The reducing power assay further confirmed the electron-donating capacity of the extract, supporting its ability to terminate free radical chain reactions. The overall antioxidant performance showed a strong correlation with the quantified phenolic and flavonoid content, highlighting the mechanistic role of these phytoconstituents. Importantly, the synergistic interaction among the three plant components contributed to a broad-spectrum antioxidant effect, targeting multiple pathways involved in oxidative cardiac injury. This multi-mechanistic action is particularly relevant in preventing lipid peroxidation, maintaining membrane integrity, and reducing oxidative burden in cardiovascular tissues. In conclusion, the polyherbal extract demonstrated significant in vitro cardioprotective potential and may serve as a promising candidate for further in vivo studies and therapeutic development targeting oxidative stress-mediated cardiovascular disorders.

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